

10/524193

Fig. 1

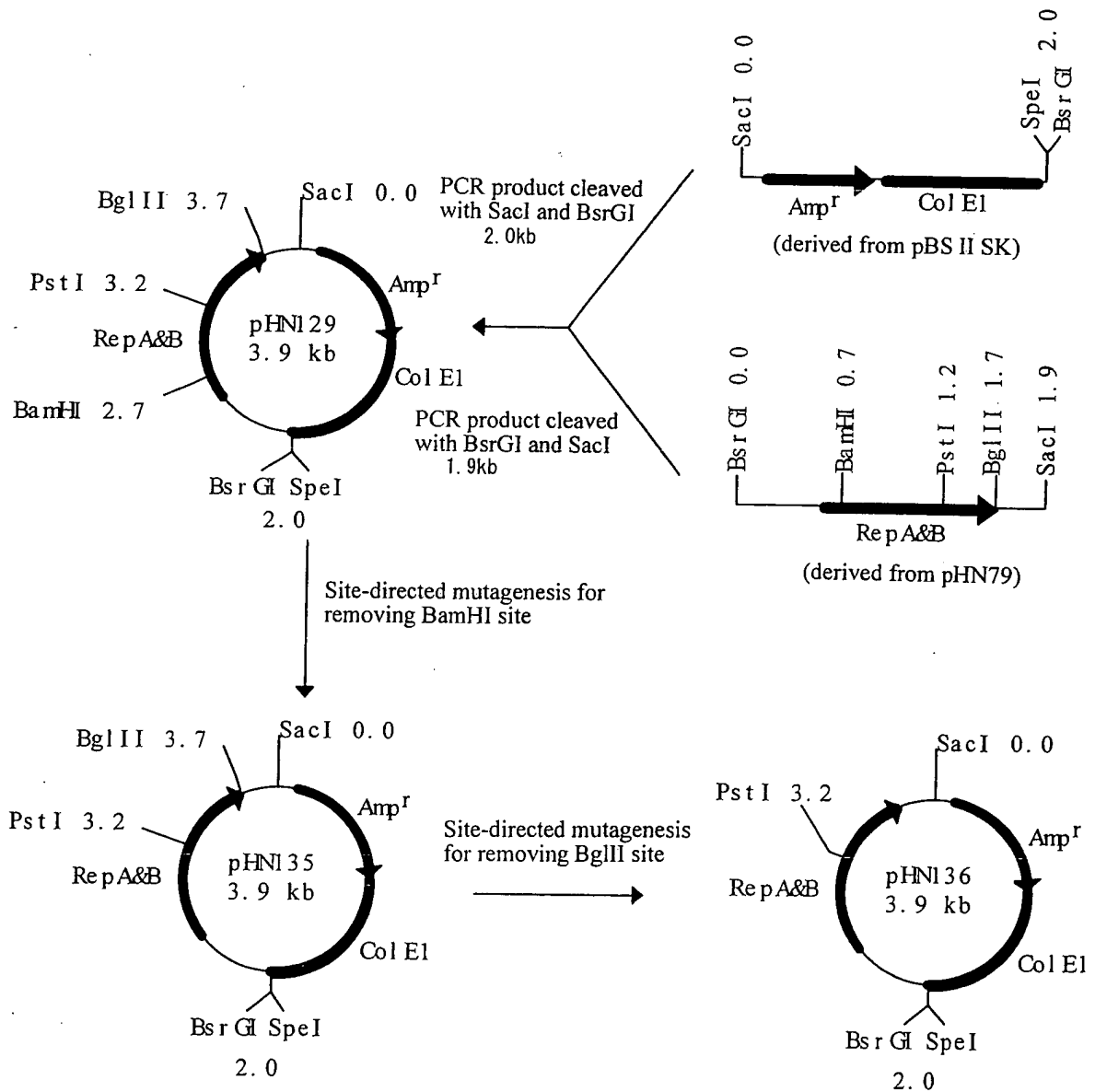


Fig. 2

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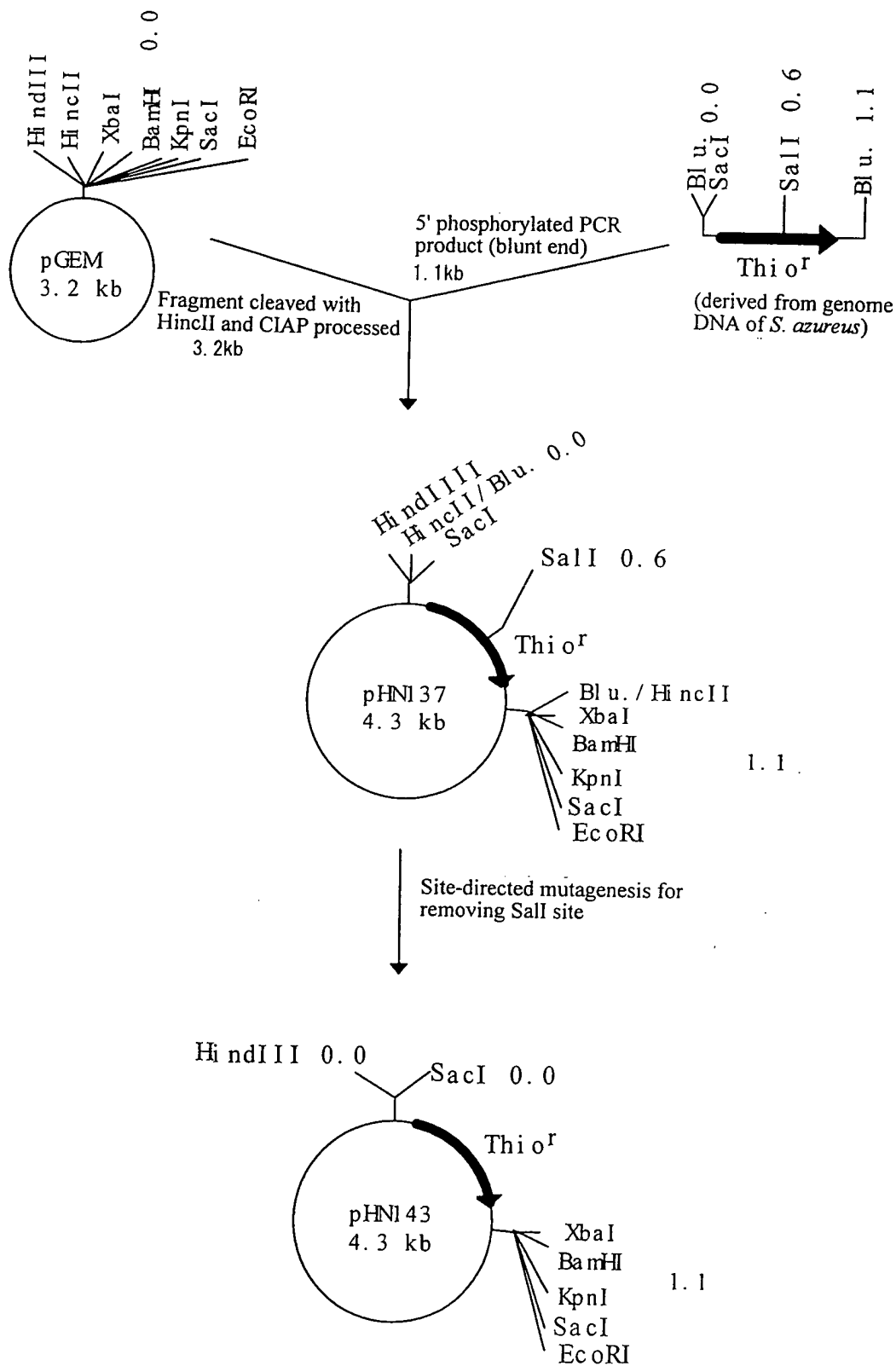
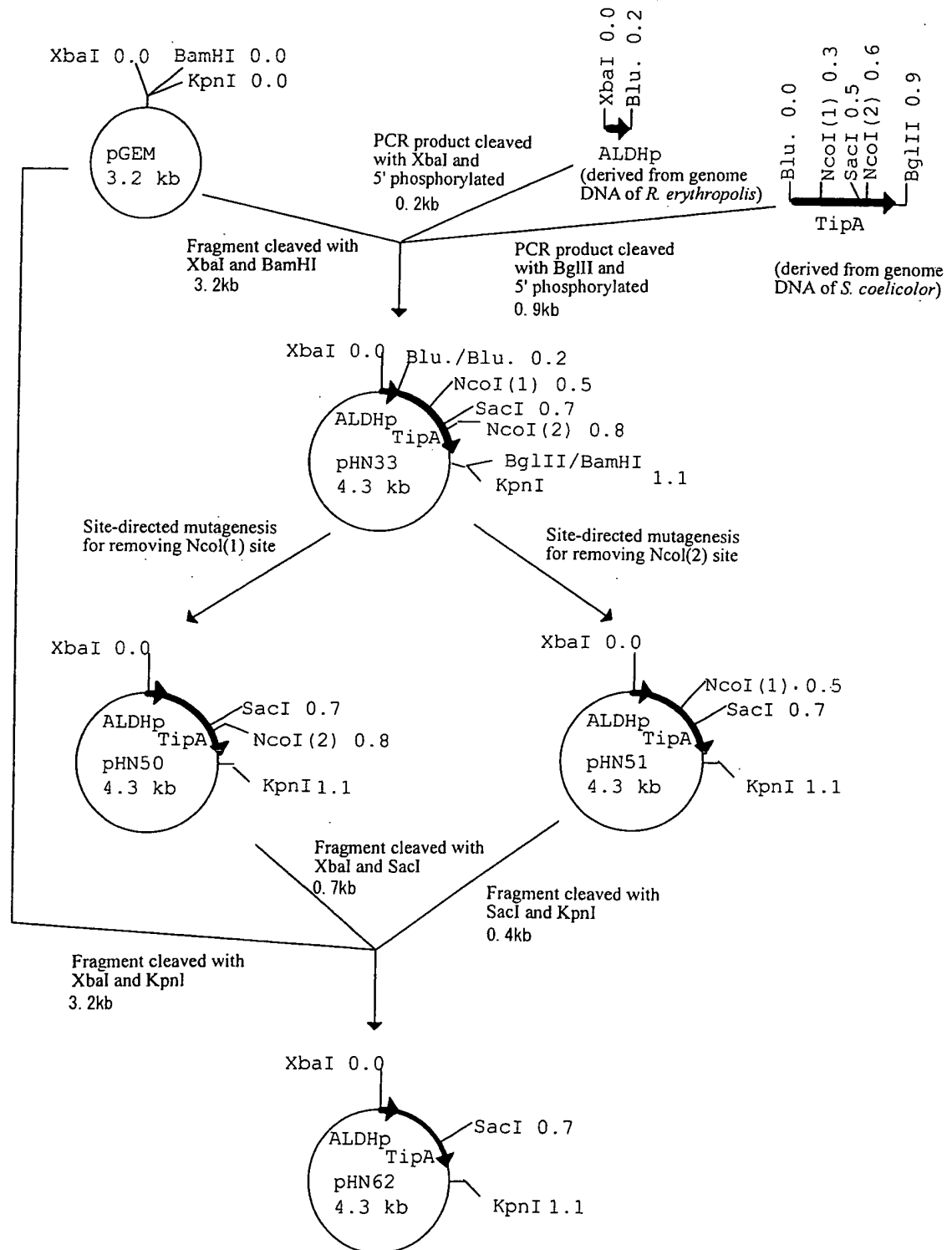


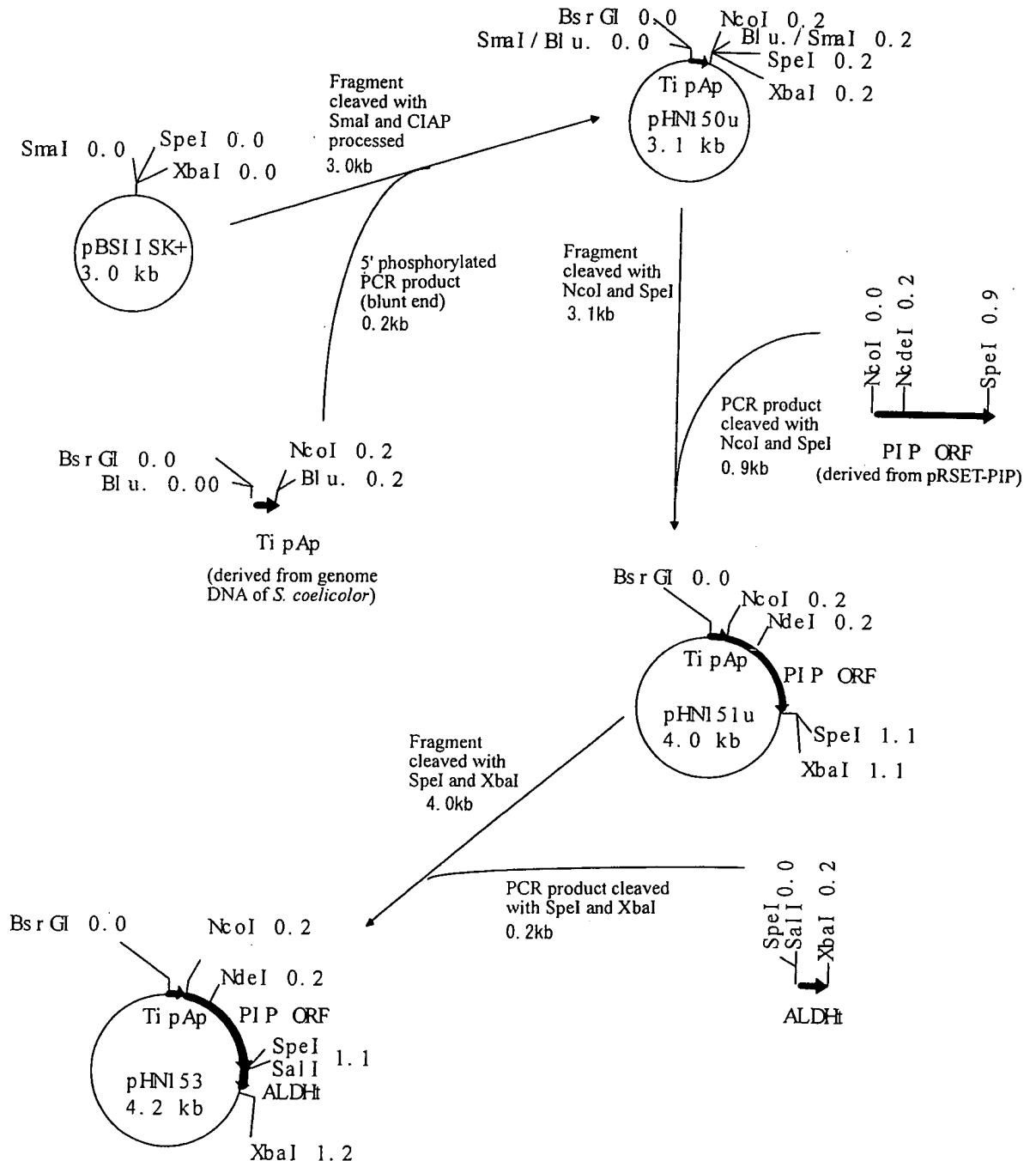
Fig. 3

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Fig. 4

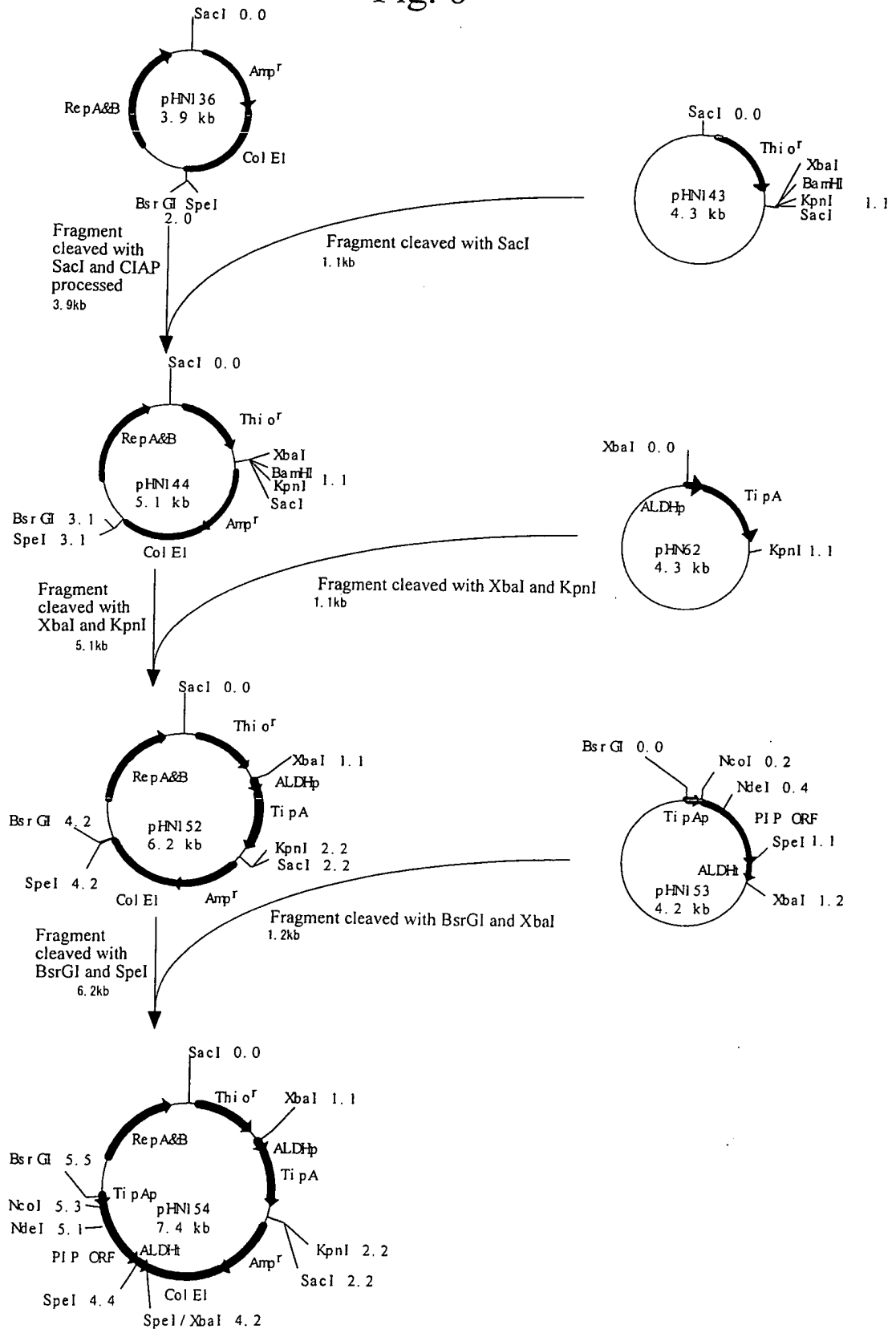


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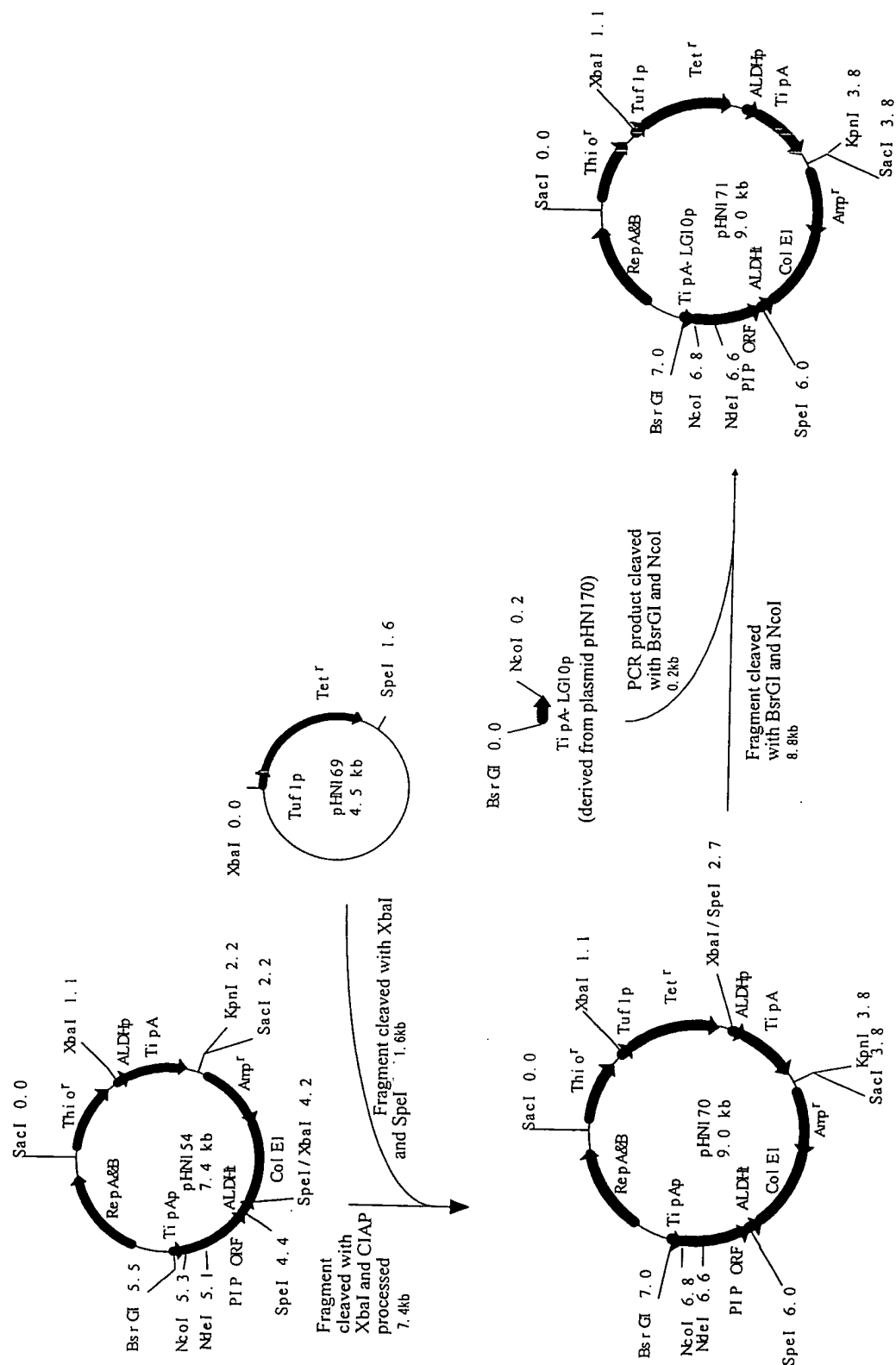
10/524193

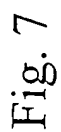
Fig. 6



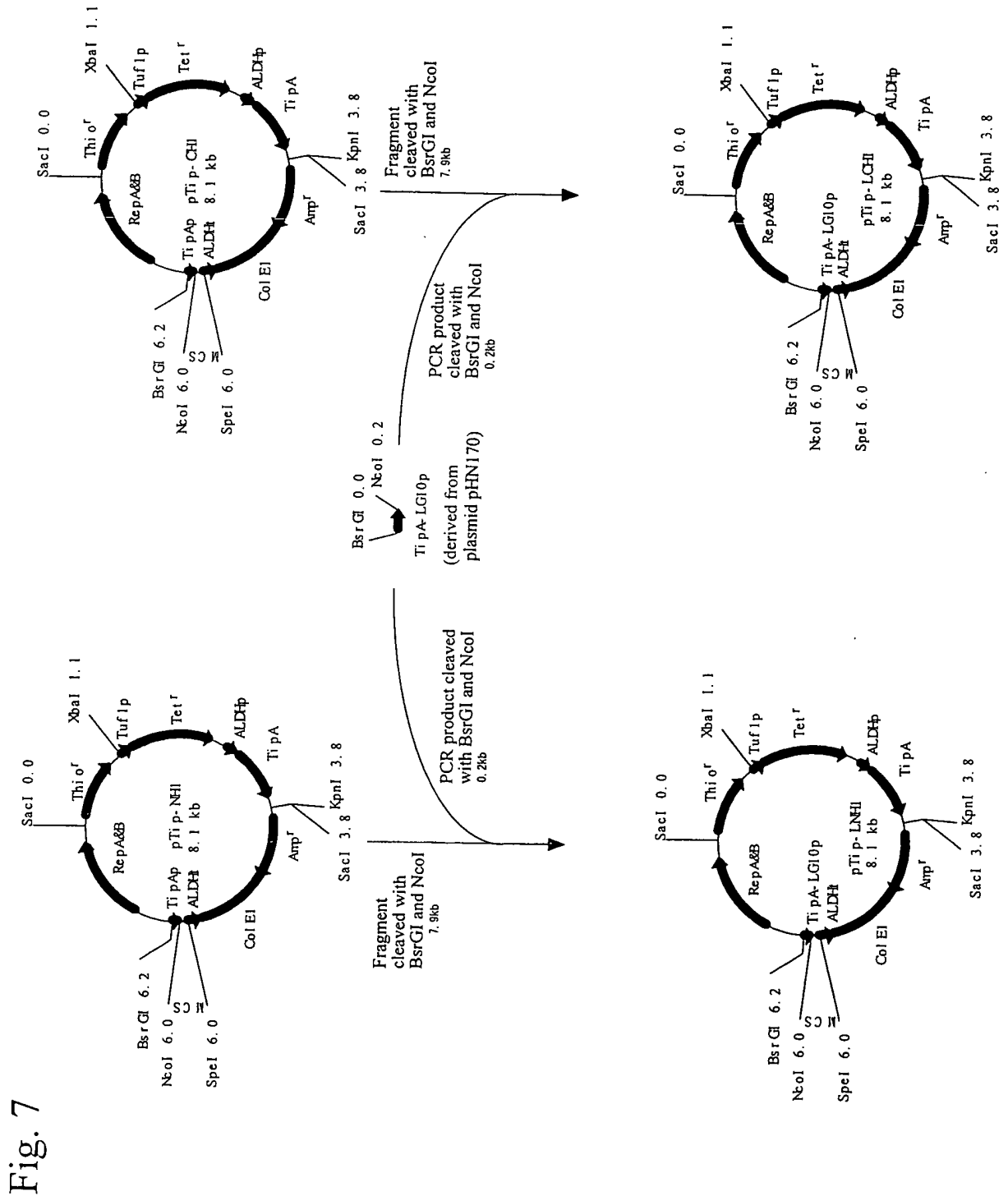
10/524193

Fig. 6



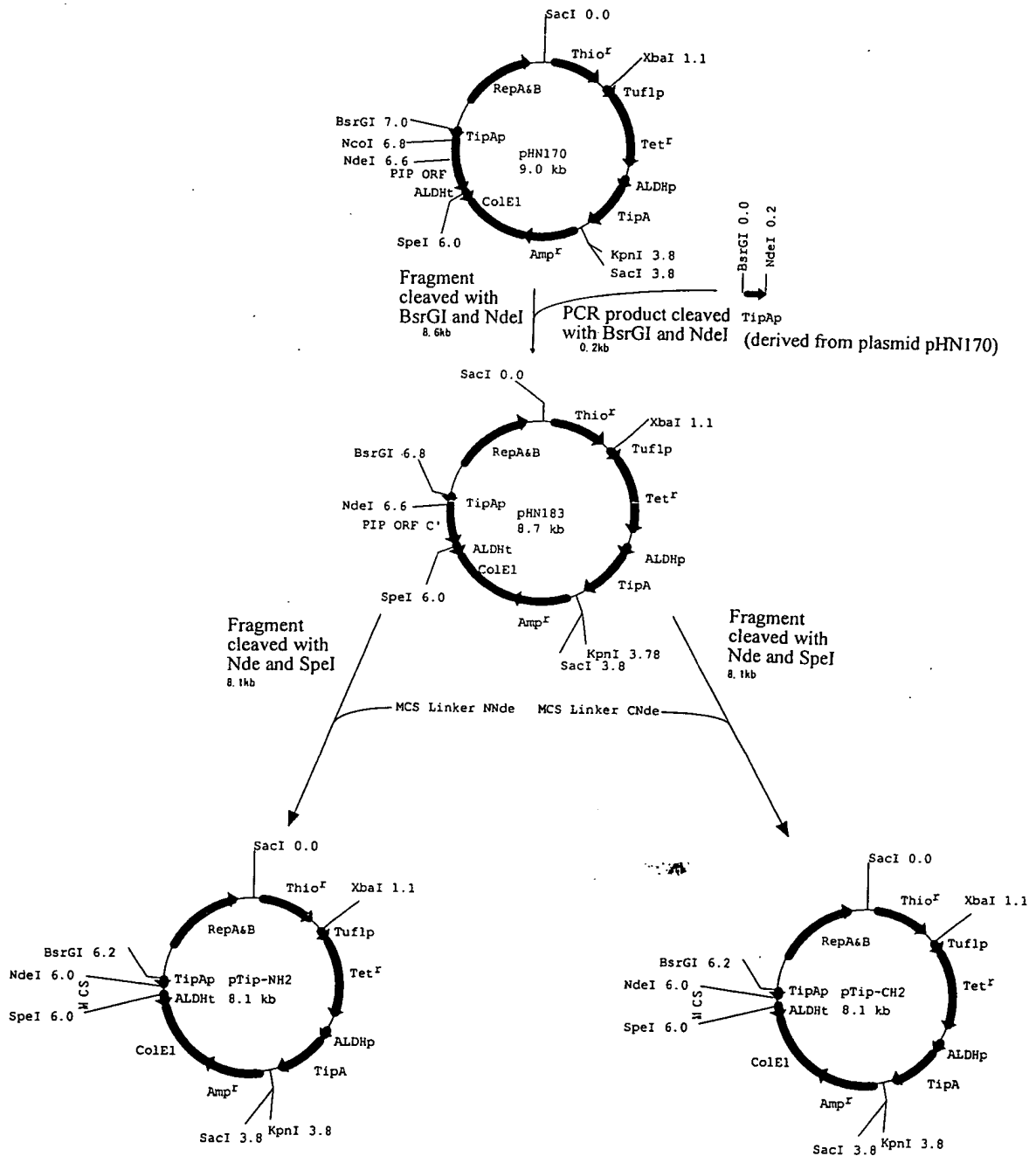


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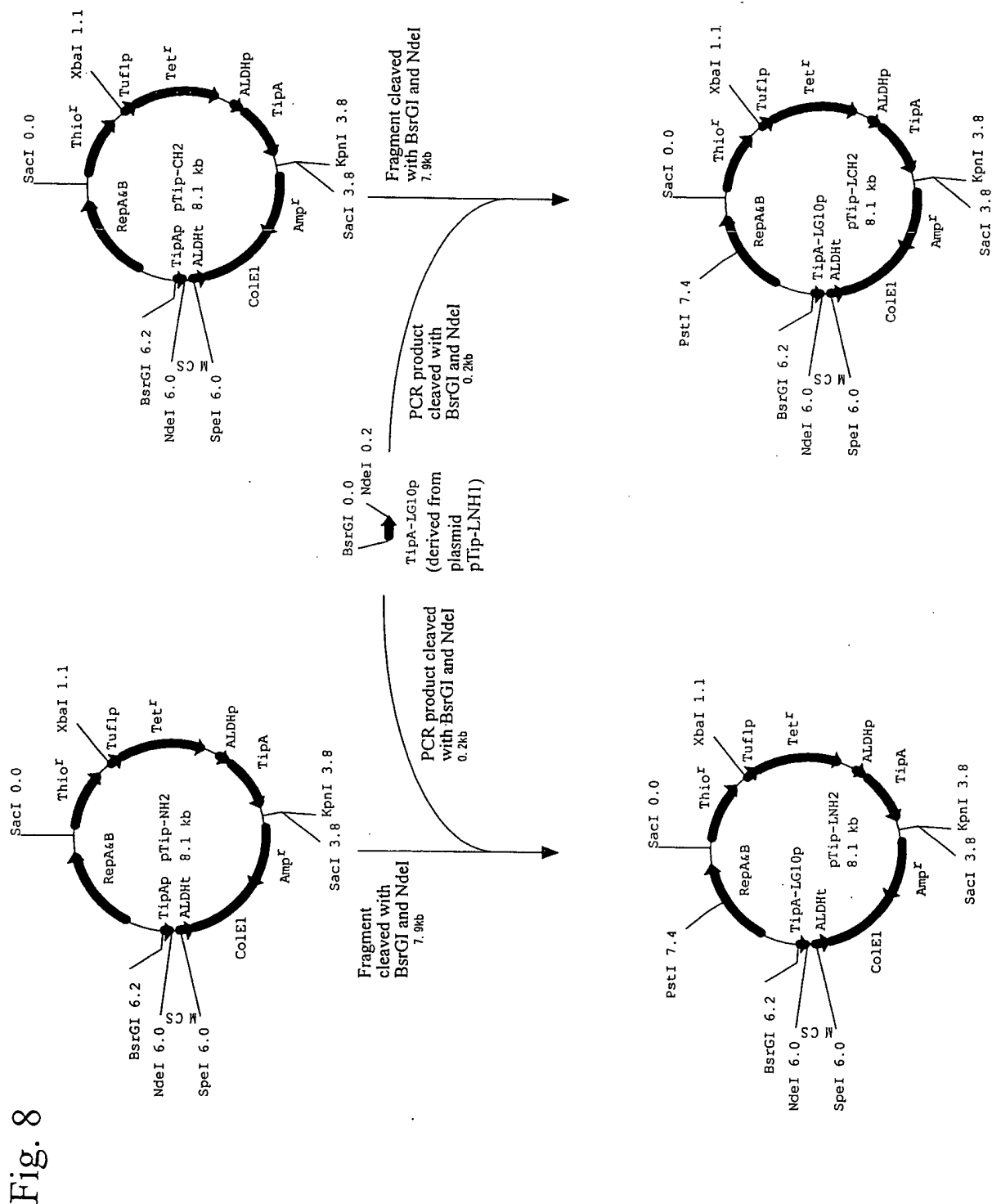


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Fig. 8



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pT p-LNH1 **pT p-LCH1** **pT p-NH1** **pT p-CH1**

pT p-LNH2 **pT p-LCH2** **pT p-NH2** **pT p-CH2**

TpA-LG10p **TpA-LG10p** **TpA-LG10p** **TpA-LG10p**

NcoI **NdeI** **NcoI** **NdeI**

(6xHis) **(6xHis)** **(6xHis)** **(6xHis)**

NdeI **NcoI** **NdeI** **NcoI**

EcoRI **EcoRI** **EcoRI** **EcoRI**

SnaBI **SnaBI** **SnaBI** **SnaBI**

NotI **NotI** **NotI** **NotI**

BamHI **BamHI** **BamHI** **BamHI**

HindIII **HindIII** **HindIII** **HindIII**

BglIII **BglIII** **BglIII** **BglIII**

XhoI **XhoI** **XhoI** **XhoI**

(Stop) **(Stop)** **(Stop)** **(Stop)**

SpeI **SpeI** **SpeI** **SpeI**

Sall **Sall** **Sall** **Sall**

ALDHt **ALDHt** **ALDHt** **ALDHt**

TpA **TpA** **TpA** **TpA**

RepA&B **Thio^r** **Tuf1p** **Tetr^r** **CoE1** **ALDHp** **TpA** **Amp^r**

MCS

pT p vector **8.1 Kb**

Thio^r = confers thiostrepton resistance to *R. erythropolis*

ALDH p = promoter which constitutively produces TipA protein

TipA = encodes a TipA protein

TipA p = TipA promoter

TipA-LG10p = improved TipA promoter

ALDH t = transcription termination sequence

Regions necessary for the autonomous replication of a plasmid

 $\text{ColE1} = \text{for } E. coli$ RepA&B = for *R. erythropolis*

Antibiotic resistance marker

Tuf1 p-Tet^r = transformation marker for *R. erythropolis*

Am p^r = transformation marker for *E. coli*

BsrGI

GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC GCG GCT GAG GGA GCC GAG CAC

GGC ACG CGG CGG CTC ACG GCG TGG CAC GCG GAA CGT CCG GGC TTG CAG CTC
-35

→ → - - - - -
ACG TCA CGT GAG GAG GCA GCG TGG ACG GCG TCA GAG AAG GGA GCG GCG ATG
-10 Met

GTC TAG AAA TAA TTT TGT TTA ACT TTA AGA AGG AGA TAT ACC

RBS

NcoI

GGC CAC CAT CAC CAT ATG GGA ATT CTA CGT AGC GGC GCG GGA TCC
Gly His His His His Met Gly Ile Leu Arg Ser Gly Arg Gly Ser

BamHI

HindIII *BglII* *XhoI* *SmaI* *EcoRI* *SnaBI* *NotI* *BamHI*

AAG CTT AGA TCT CGA GGA TGA ACT AGT CGA CCC ACC GGC ACC CGT GAG CCC
Lys Leu Arg Ser Arg Gly *

→

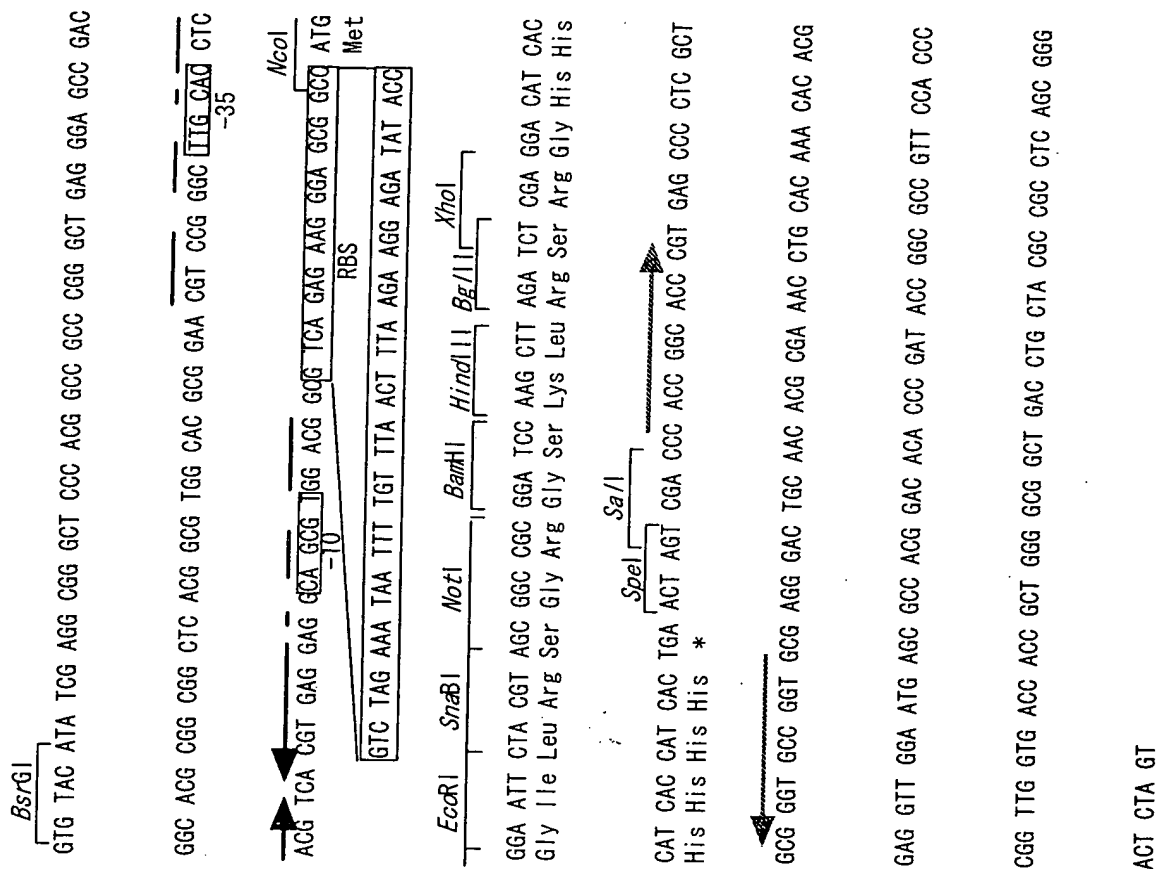
CTC GCT GCG GGT GCC GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA

CAC ACG GAG GTT GGA ATG AGC GCG ACC GAC ACA CCC GAT ACC GGC GCC GTT

CCA CCC CGG TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC CTC

AGC GGG ACT CTA GT

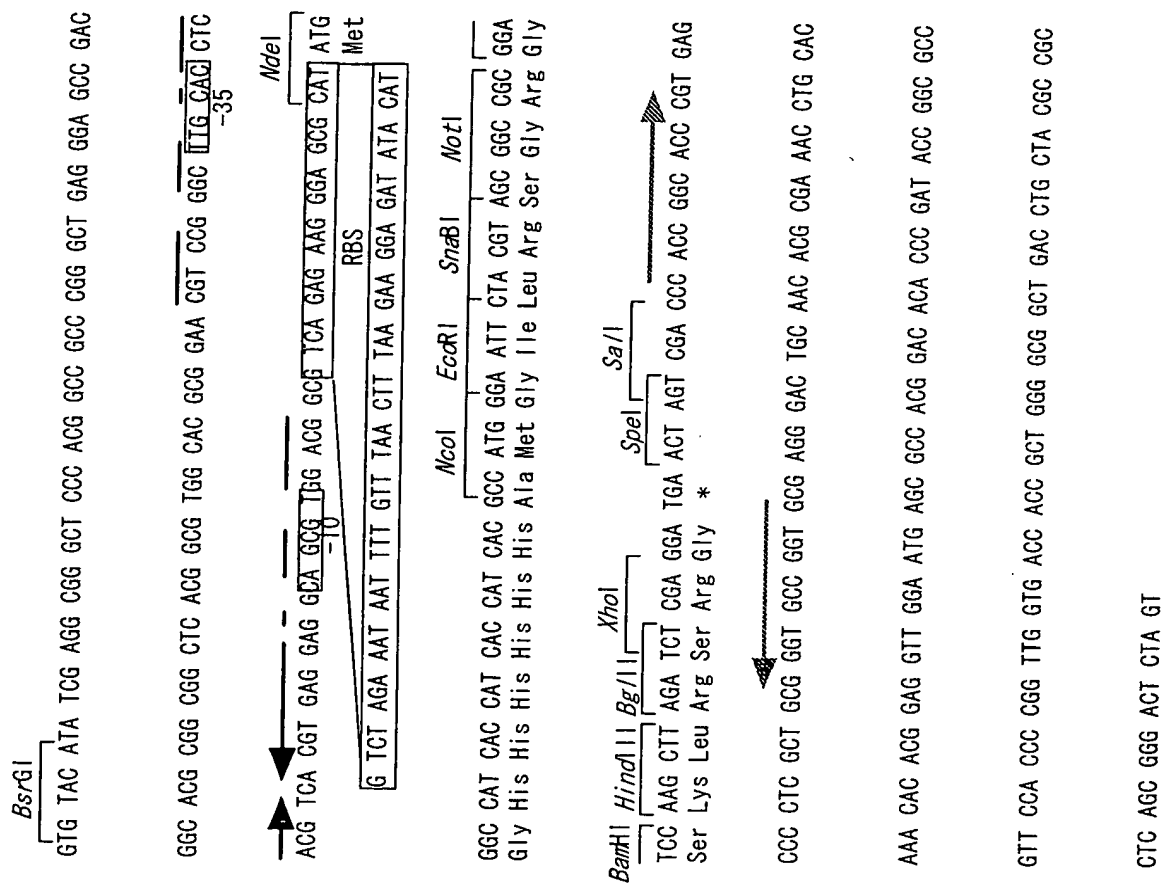
Fig. 9c



Title: Novel expression vector suitable
for expression of recombinant protein
at low temperature
Inventors: Nakashima et al.
Atty. Dkt. No.: 081356-0232

10/524193

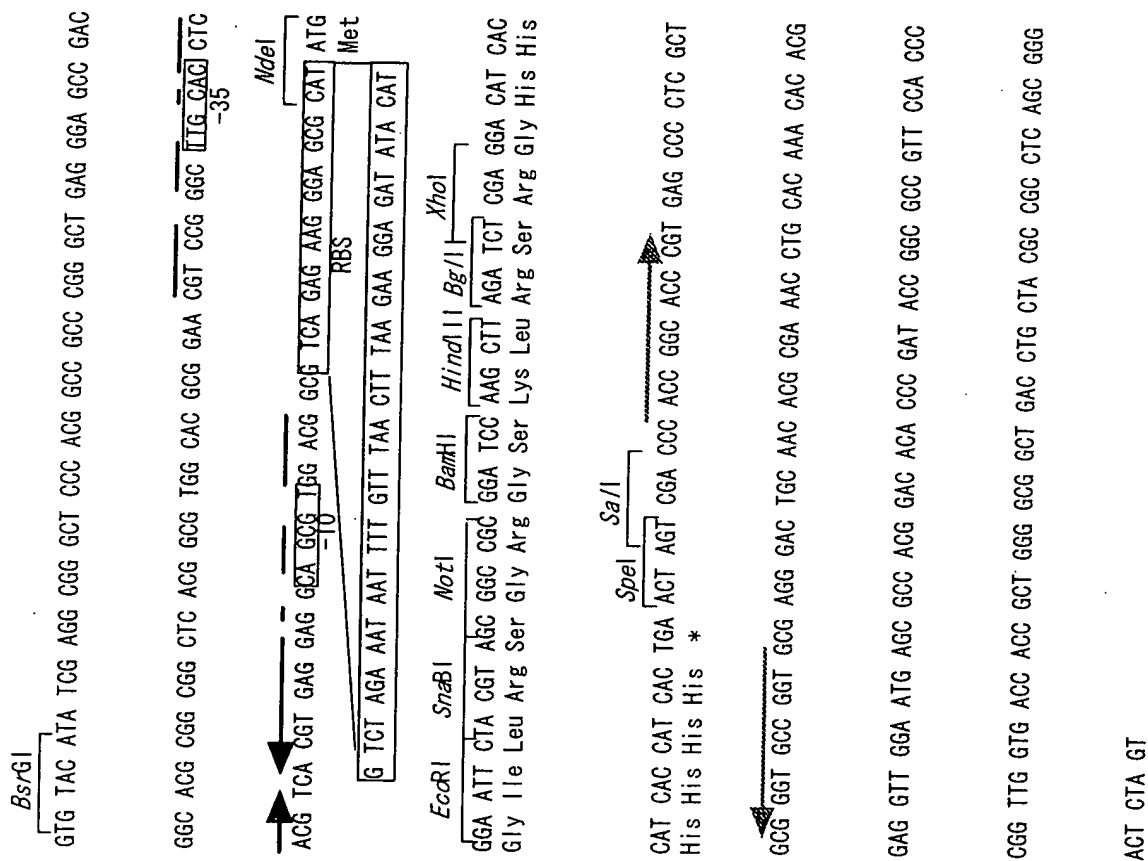
Fig. 9d



Title: Novel expression vector suitable
for expression of recombinant protein
at low temperature
Inventors: Nakashima et al.
Atty. Dkt. No.: 081356-0232

10/524193

Fig. 9e

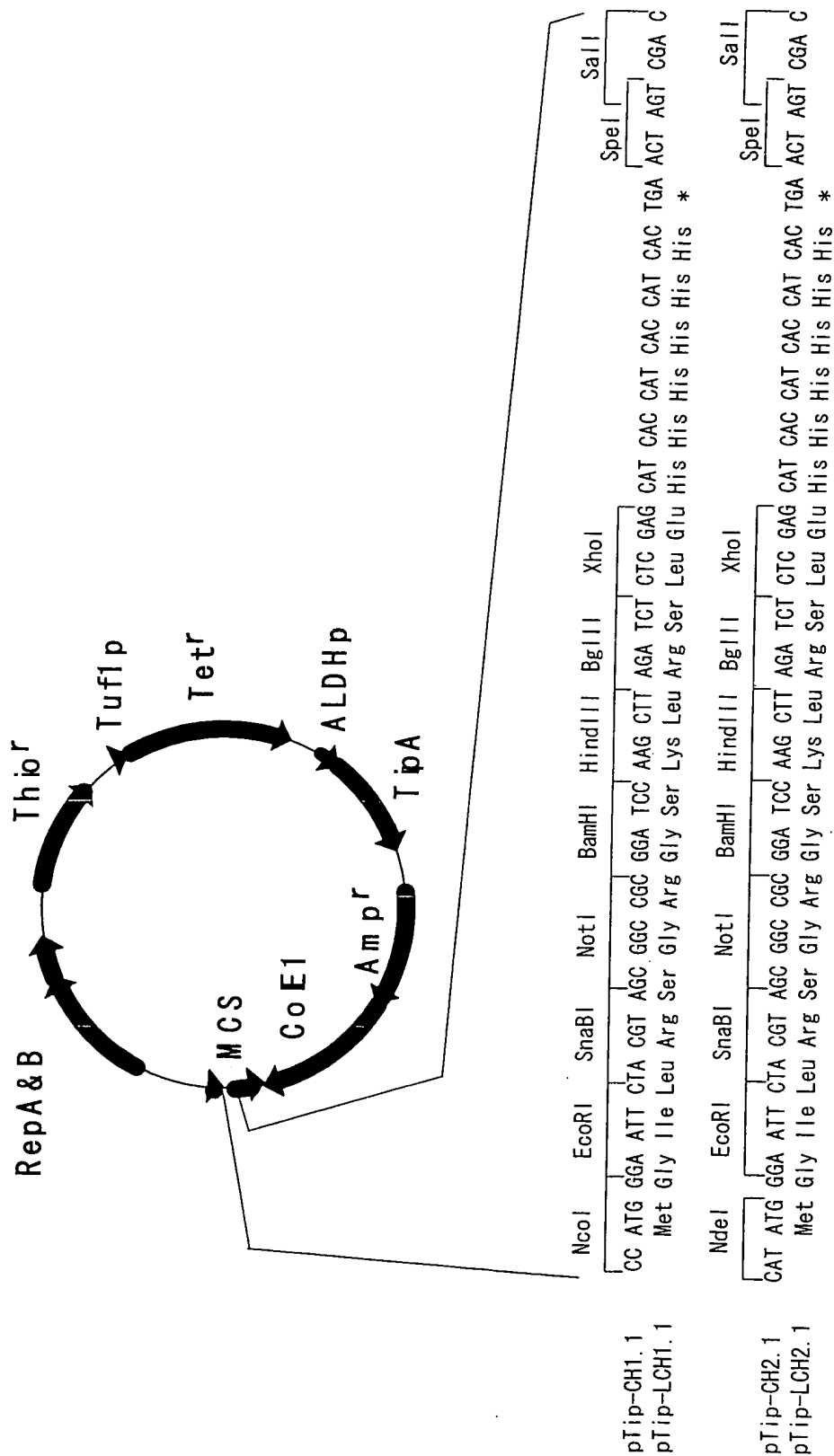


Title: Novel expression vector suitable
for expression of recombinant protein
at low temperature
Inventors: Nakashima et al.
Atty. Dkt. No.: 081356-0232

10/524193

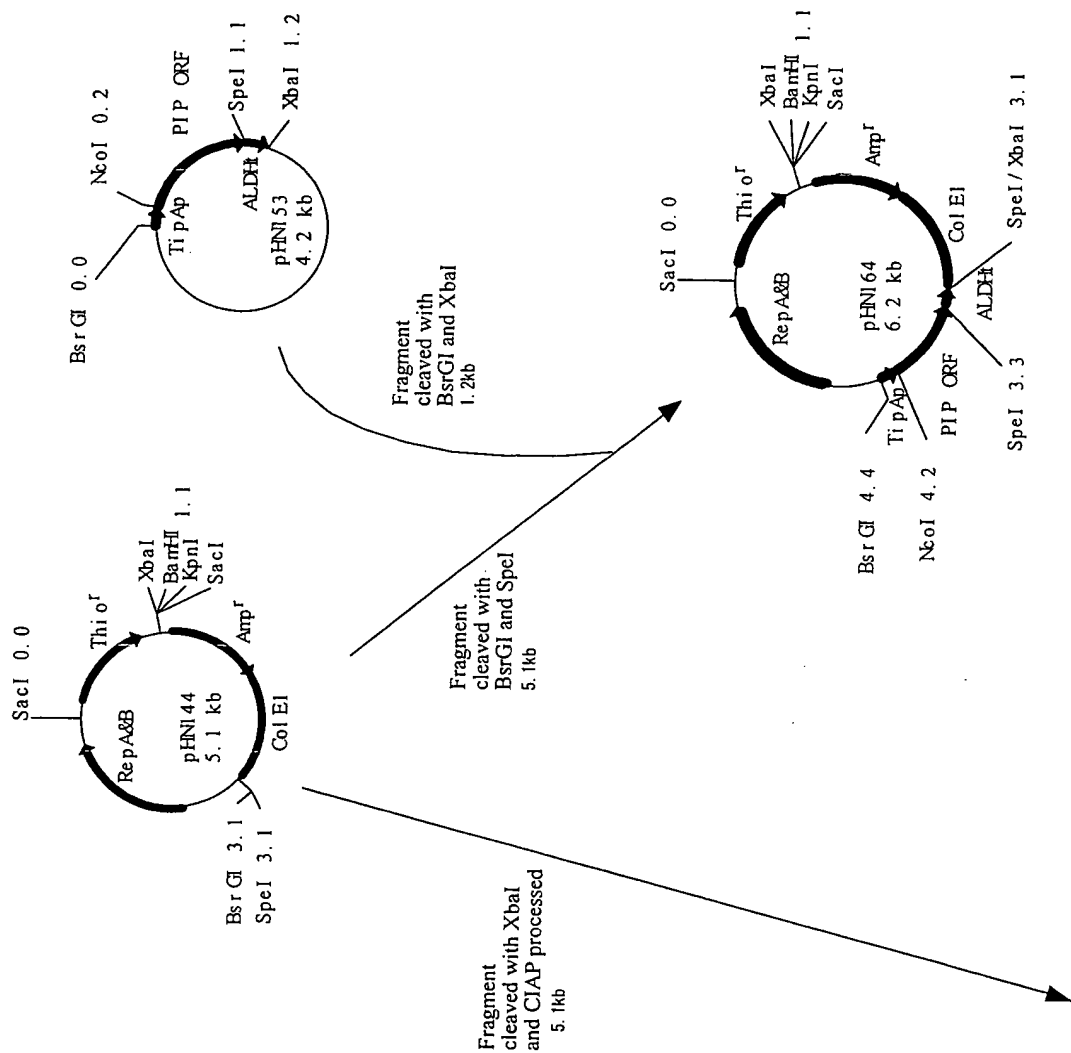
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Fig. 10



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Fig. 11



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Fig. 11

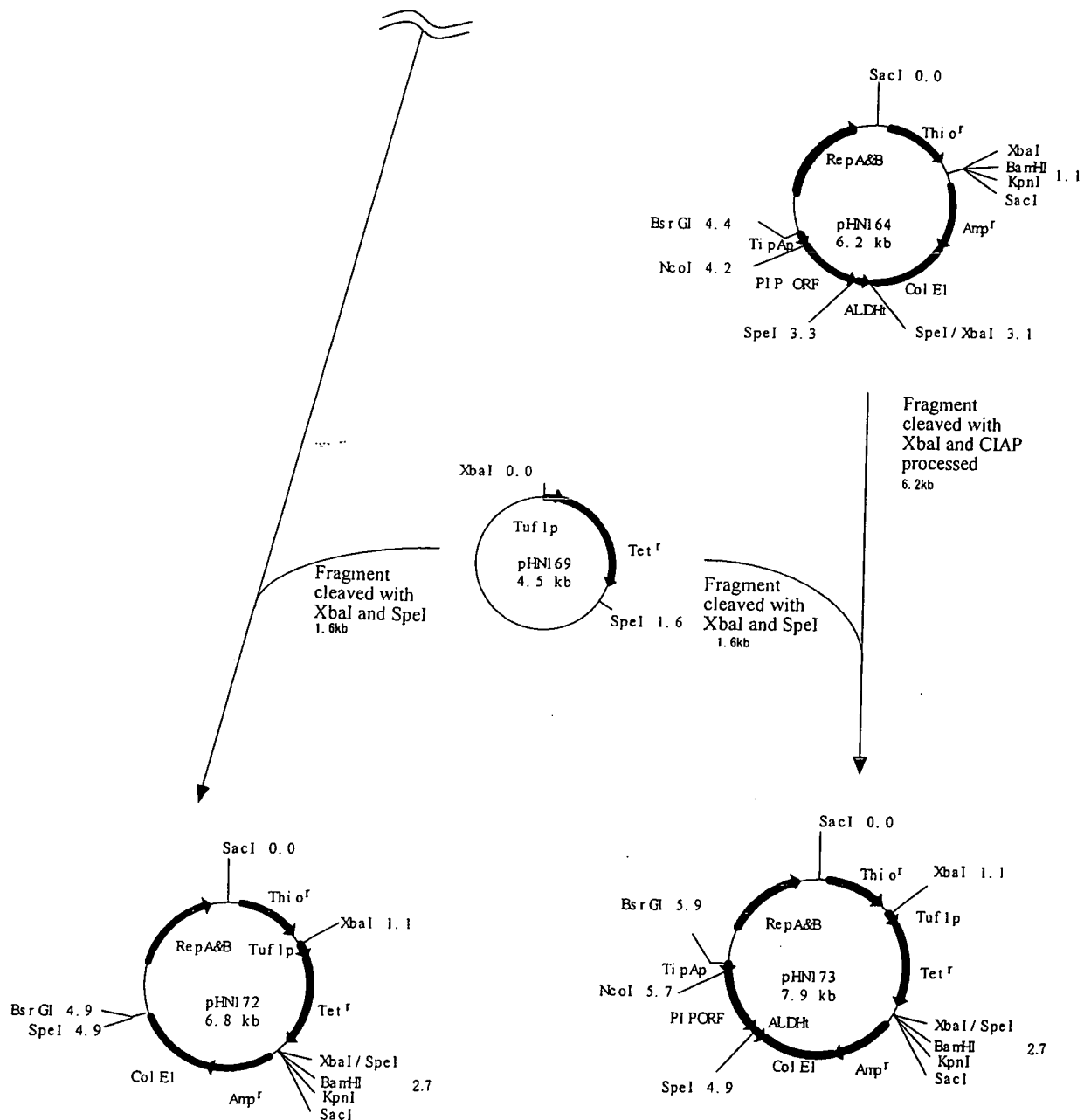
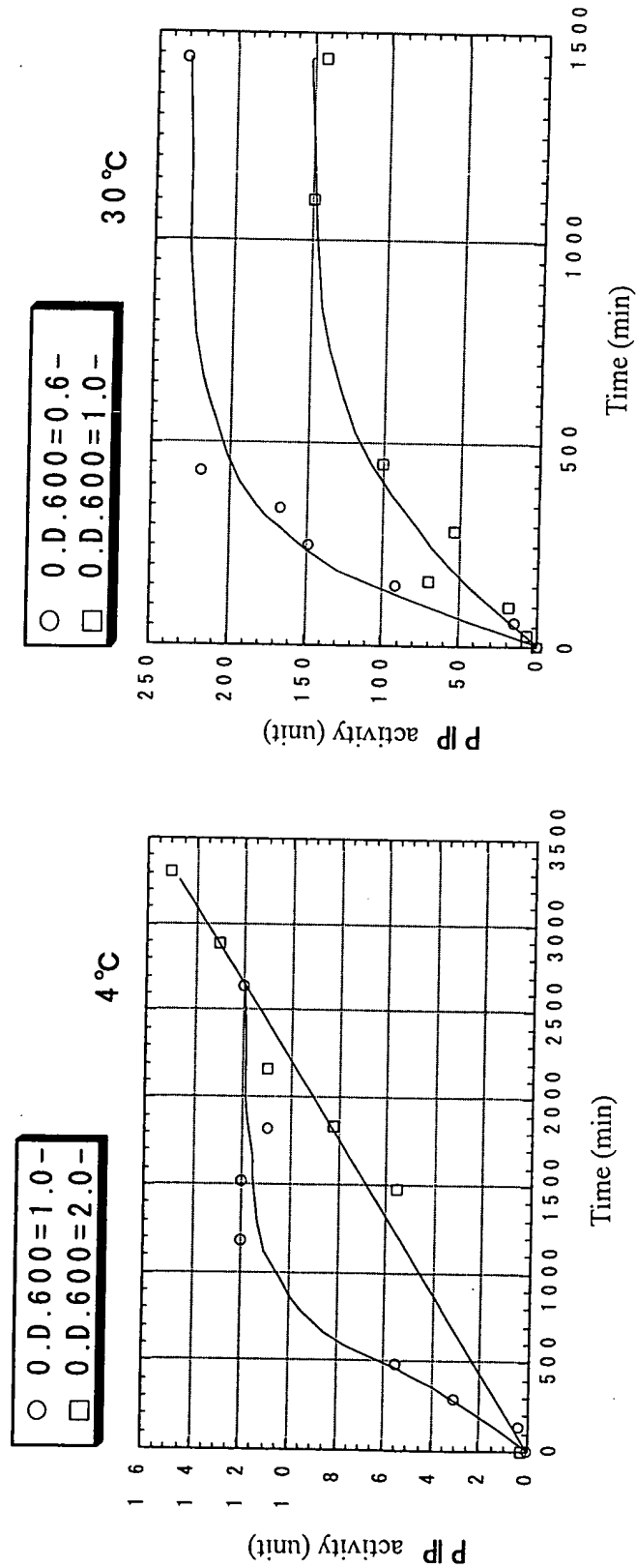


Fig. 12

1µg/ml Thiostrepton	Activity (+/-Thiostrepton) (unit)	Culturing Temperature (°C)	Culturing volume (µl)	Plasmid used for transformation of <i>R. erythropolis</i>	Inducer cassette		Expression cassette			
					ALDHP	TiPA	TiPAp	PIP	ORF	ALDHT
+	-									
	16/0.5	4°C	5	pHN170	+	+	+	+	+	+
	0.1/0.2	4°C	5	pHN173	-	-	+	+	+	+
	0.1/0.1	4°C	5	pHN172	-	-	-	-	-	-
	241/4	30°C	0.5	pHN170	+	+	+	+	+	+
	0.9/0.6	30°C	0.5	pHN173	-	-	+	+	+	+
	0.3/0.3	30°C	0.5	pHN172	-	-	-	-	-	-

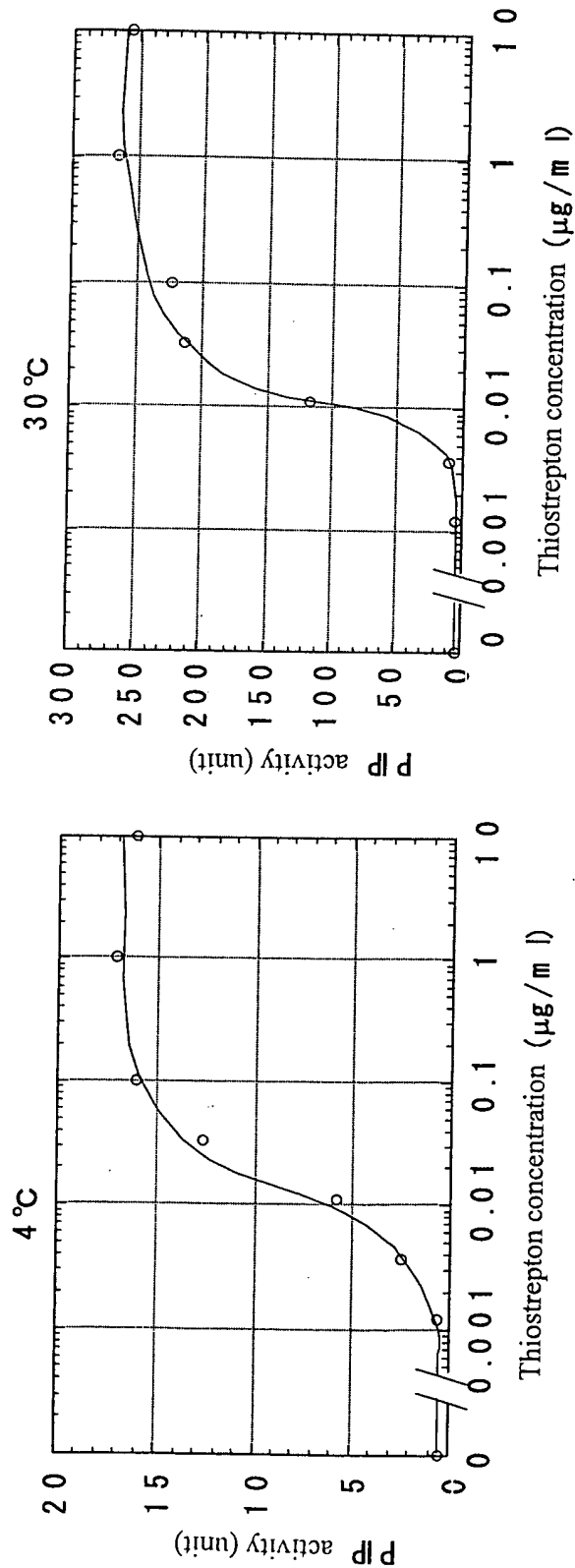
10/524193

Fig. 13



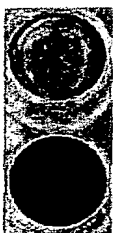



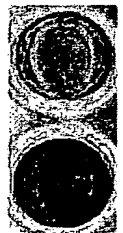
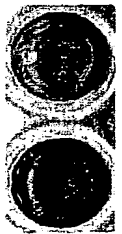
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Fig. 14



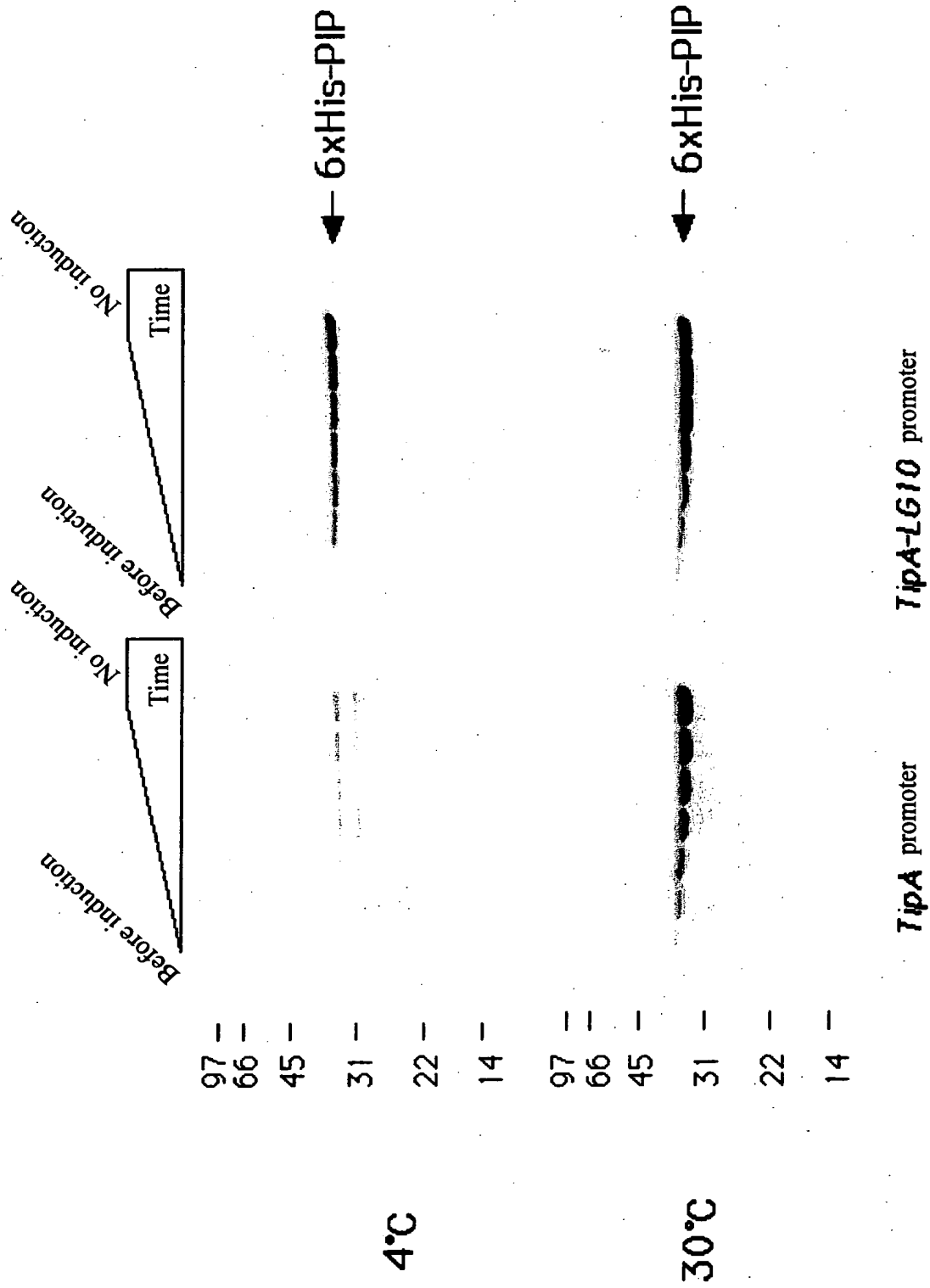
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Fig. 15

1 μ g/ml Thiostrepton	Activity (+/-Thiostrepton) (unit)	Culturing Temperature (°C)	Host strain transformed with pHN170	Culturing volume (μ l)
+				
-				
	13/0.8	4°C	<i>R.erythropolis</i>	20
	7/0.8	4°C	<i>R.fascians</i>	20
	1.9/0.3	4°C	<i>R.opacus</i>	100
	215/2	30°C	<i>R.erythropolis</i>	2.5
	34/0.4	30°C	<i>R.fascians</i>	2.5
	6/1	30°C	<i>R.opacus</i>	20

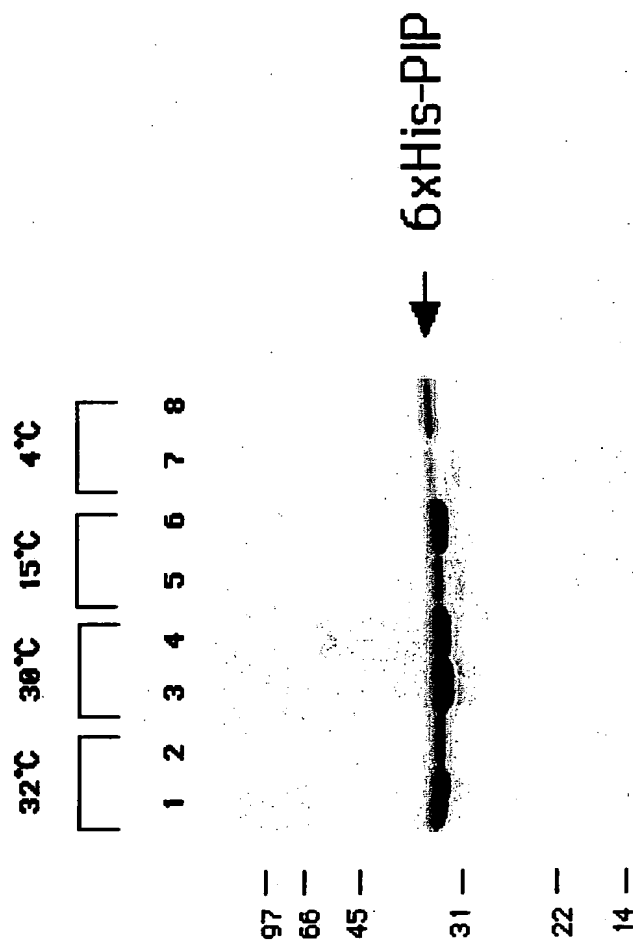
10/524193

Fig. 16



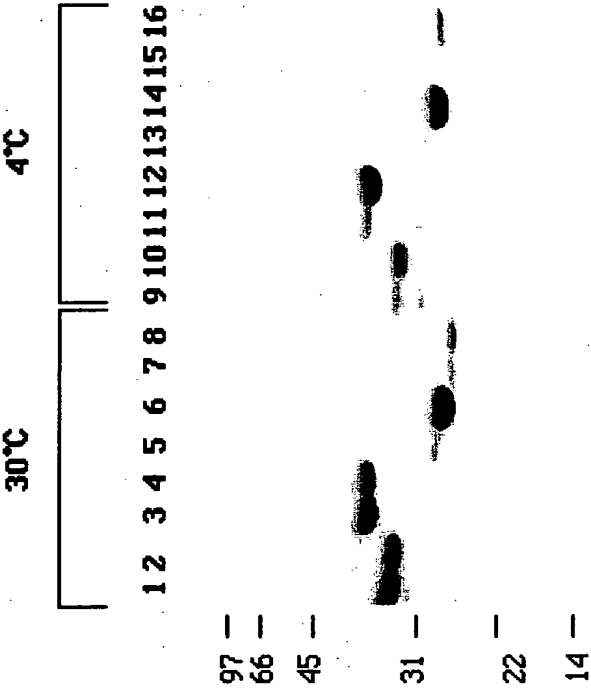
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Fig. 17



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Fig. 18



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Fig. 19

Temperature	Reporter	WT	LG10	Magnification (LG10/WT)
30°C	PIP	11	6.3	0.57
	AtPIP	11	4.6	0.39
	GFP	1.1	10	9.1
	GST	0.16	1.3	8.1
4°C	PIP	0.29	2.6	8.9
	AtPIP	0.13	2.9	22
	GFP	<0.01	3.9	>390
	GST	<0.01	1.3	>130

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Fig. 20

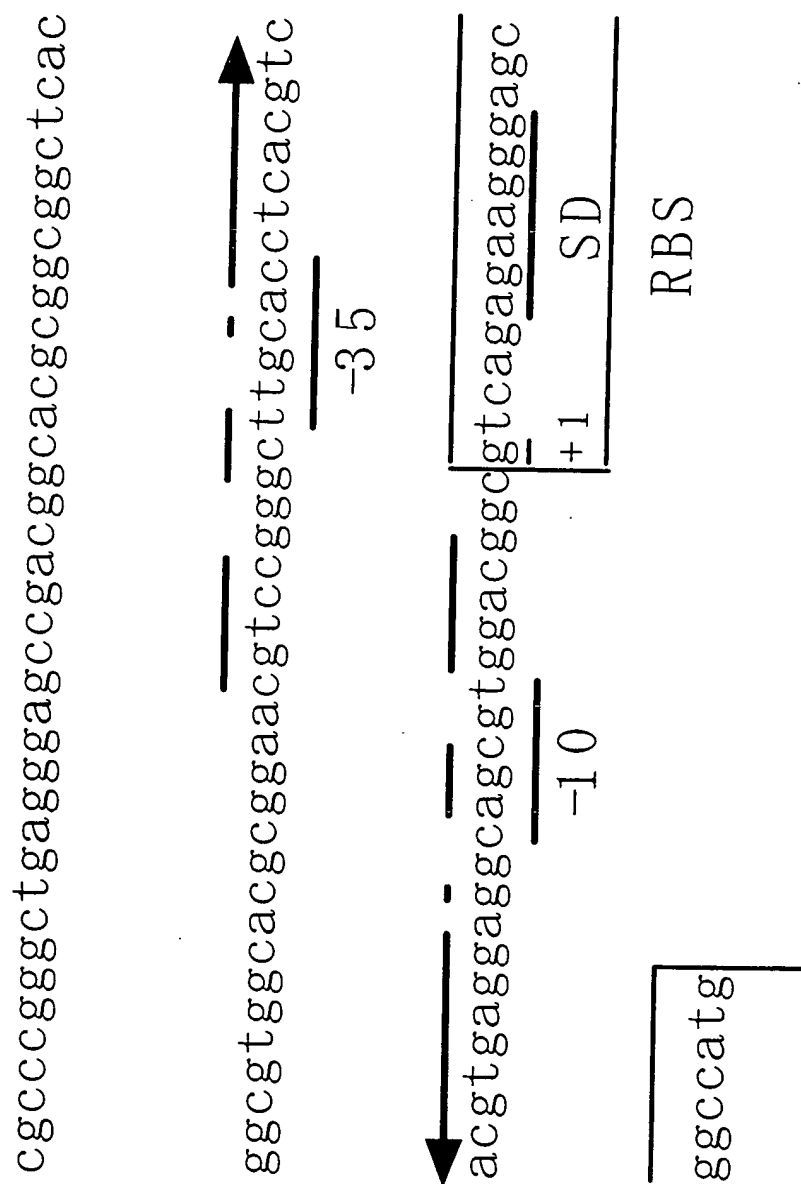
Name of plasmid	Class	GenBank Accession No.	Type	Number
	Functionally known protein (two or more obtained)		6	16
LE20	Serum amyloid A (Saa1)	M11131		6
L113	NADH dehydrogenase 1 α 4	BC011114		2
LE59	Pantotenate kinase 1 β	AF200357		2
LE94	Retinol binding protein 4 (RBP4)	AK008765		2
LE98	Major urinary protein 4 like	BC019965		2
LE287	Histidine-rich glycoprotein	NM_053176		2
	Functionally unknown protein (singly obtained)		24	24
L3	Cytochrome b5 like	AK002426		1
LE2	Fibrinogen A alpha	BC005467		1
LE3	Clusterin	NM_013492		1
LE9	Splicing factor 3b subunit 1 155kDa	NM_031179		1
LE12	Haptoglobin	NM_017370		1
LE18	Peroxiredoxin 4	BC019578		1
LE82	Inter-alpha-trypsin inhibitor Heavy Chain 2	NM_010582		1
LE87	RIKEN130000F09, Highly similar to VIP36	NM_025828		1
LE95	Serum albumin	AJ011413		1
LE125	Arylacetate deacetylase	BC019999		1
LE137	New cDNA, Highly similar to UDP-Glycosyltransferase	-		1
LE156	RIKEN1300017J02, Highly similar to Transferrin	AK005035		1
LE171	Phosphatidylinositol 3-kinase	NM_008839		1
LE178	Protein kinase C receptor (RACK1) like	D29802		1
LE204	EGF receptor	AF275367		1
LE247	Retinoic acid receptor responsive protein TIG2	AK002298		1
LE251	Insulin-like growth factor IA	X04480		1
LE280	Transferrin	BC022986		1
LE295	Apolipoprotein A-V	NM_080434		1
LE305	Fatty Acid Binding Protein 1 (FABP1)	BC009812		1
LE354	Retinoblastoma binding protein 7 (Rbbp7)	NM_009031		1
LE357	Zinc fingers and homeoboxes protein 1 (Zhx1)	NM_009572		1
LE416	Tumor differentially expressed 1 like (Tde1l)	NM_019760		1
LE421	RIKEN1300006C19, Highly similar to OSTSTT3	AK018758		1
	Functionally unknown protein		4	4
LE25	IMAGE:4239007, DUF92 like membrane Protein?	BC016895		1
LE51	New cDNA, No homology	-		1
LE119	IMAGE:3489640, Bone marrow stromal protein like?	BC008532		1
LE123	RIKEN1500015G18, No homology	NM_025439		1
	Subtotal		34	44
	Other proteins (out of ORF or not important proteins)			382
	Total			426

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Fig. 21

category	Protein	6xHis tag	Presumed molecular weight	<i>R. erythropolis</i>				Name of plasmid	Name of plasmid	<i>E. coli</i>	
				Sup/Ppt	Proliferation	30 °C	4 °C			Sup/Ppt	30 °C
Protein isolated by screening	Saa1	N'	12 (14)	0.4/1	-	0.08/2	-	pHN205	pHN193	N.D./N.D.	+-
	NADH4	N'	9	N.D./0.2	+	N.D./0.2	+	pHN206	pHN195	N.D./N.D.	++
	Cytochrome b5	N'	15	0.2/8	+-	0.5/4	+	pHN208	pHN199	N.D./0.8	+
	LE123	N'	19 (21)	0.04/0.08	+	0.03/0.06	+	pHN287	pHN276	N.D./N.D.	+-
	Transferrin	N'	75 (77)	0.2/0.5	+	0.06/0.2	+	pHN289	pHN277	0.2/0.2	++
	Apoa5	N'	39 (41)	3/8	+-	2/4	+	pHN288	pHN281	N.D./N.D.	+++
	PanK	N'	42						pHN279	2/N.D.	++
	Peroxyredoxin4	N'	27 (31)						pHN278	4/0.4	++
	TFL	N'	75 (77)						pHN280	0.2/0.2	++
Insoluble protease	Cathepsin D	C'	43 (45)	2/3	++	0.3/2	++	pHN270	pHN273	N.D./N.D.	+++
	Prothrombin	C'	30 (70)	N.D./N.D.	+-	N.D./N.D.	+	pHN271			
	Kallikrein6	C'	26 (29)	0.3/0.3	+++	0.3/2	++	pHN272	pHN275	N.D./N.D.	+++
DNase	LSNase	N'	36 (33)	N.D./N.D.	+	N.D./N.D.	+	pHN299			
	DLAD	N'	38 (41)	N.D./N.D.	+-	N.D./N.D.	+	pHN284			
Protein inhibiting cell proliferation	HMG-1	N'	25	4/0.2	-	2/0.06	-	pHN285	pHN305	0.2/0.1	-
	Kid1	N'	66	N.D./0.08	-	N.D./0.2	-	pHN286			
	Bax alpha	N'	21	N.D./N.D.	-	N.D./N.D.	-	pHN217	pHN212	N.D./N.D.	-
Protein solubilized with low temperature dependence	Glucokinase	N'	52	4/2	++	6/2	++	pHN298	pHN306	5/1	+++
	p37A	C'	38	4/0.2	+++	3/0.1	+++	pHN291	pHN308	4/N.D.	+++
Positive control	PIP	C'	33	6/0.7	+++	3/0.3	+++	pHN171			
	LacZ	N'	120						pBAD/HisA/lac4/0.5		+++

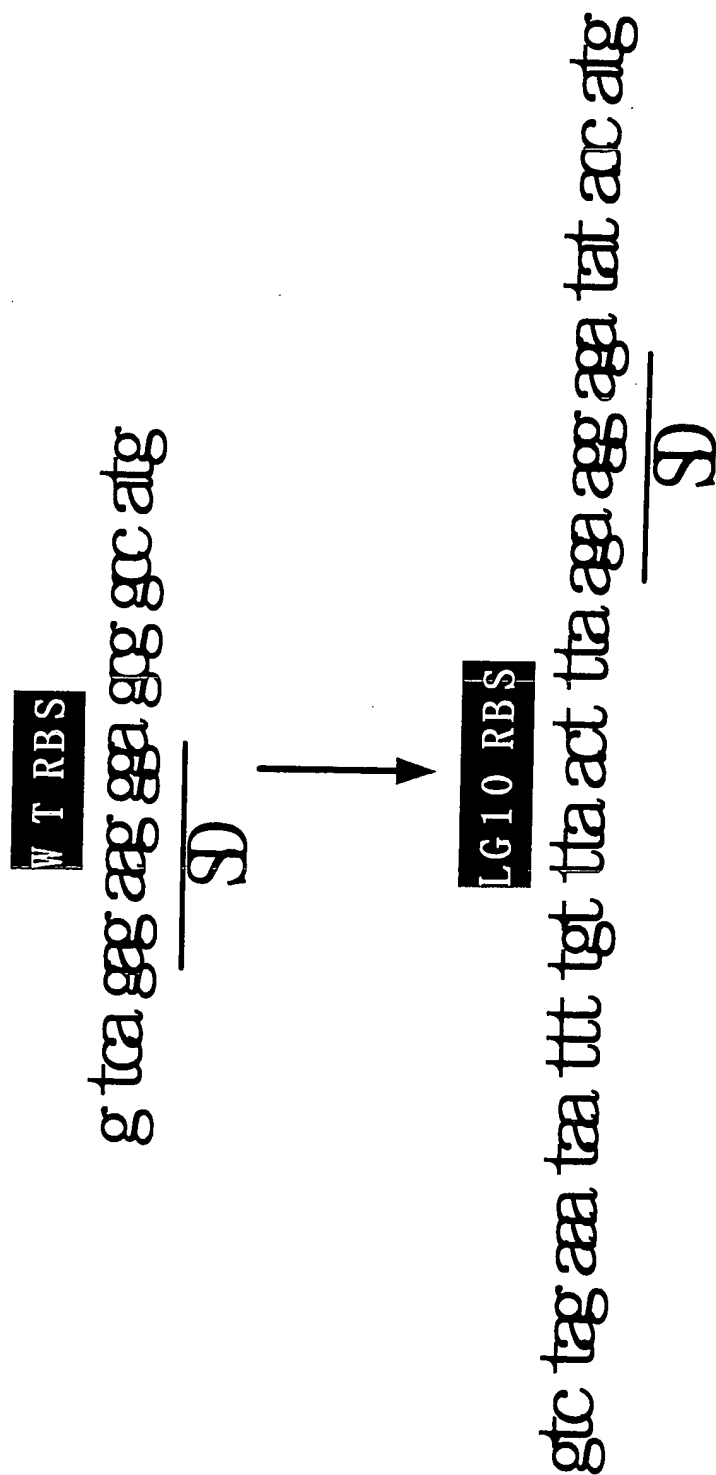
Fig. 22



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 at low temperature
 Inventors: Nakashima et al.
 Atty. Dkt. No.: 081356-0232

10/524193

Fig. 23



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Inventors: Nakashima et al.

Atty. Dkt. No.: 081356-0232

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